OBSERVATIONS ON THE BACTERIOLOGY OF INFLUENZA

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These observations were made on (A) patients affected in an epidemic in the Student Army Training Corps of the University of Chicago which I have described elsewhere;† (B) civilian influenza patients in various hospitals in the vicinity of the University of Chicago; (C) patients, mainly University students, who developed influenza in December, 1918-February, 1919, after the main Chicago epidemic had subsided, and (D) cases of tonsillitis, "colds," and other respiratory tract affections occurring during and subsequent to the epidemic. An attempt was made in Groups A, C and D to study a few cases in great detail, making frequent and very thorough bacteriologic examinations. In one case of influenza pneumonia, for example, observations extended over a period of 36 days until convalescence was complete and the patient discharged, and included sets of plate cultures on 25 different days.

One object especially in view was the determination of the relative frequency and abundance of the Pfeiffer bacillus in the upper respiratory tract of persons suffering from influenza and from common, nonspecific respiratory tract infections. Another was a series of similar observations on the diplo-streptococcus described by Mathers.[1] Variations in the nose and throat flora throughout the illness of one and the same individual were also particularly noted.

Methods.—In the majority of cases swabs have been made from the nose, tonsils and nasopharynx; the nasopharynx swabs have been obtained by the Mathers' bent wire method as used in meningococcus-carrier work.[2] Nasal swabs usually failed to give results materially different from the others and in the later cases were omitted.

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† Proc. Inst. of Med. of Chicago, 1919, 2, 135.

[1] Jour. Amer. Med. Assn., 1918, 71, p. 1733.

[2] "Standard Technique of Meningococcus Carrier Detection," published by the Medical Depts. of the Army and Navy and the U. S. Public Health Service.

The swab has been smeared as soon as made on freshly poured warm blood-agar plates, which are kept warm en route to the incubator. The meat infusion agar has been prepared with 2% peptone and made neutral to phenolphthalein; it has proved well adapted to the growth of pure cultures of the Pfeiffer bacillus. Human blood and sheep blood (5%) have been chiefly used; no material difference was noted. From the smear radiating streaks are made with a platinum loop[2] and crossed after burning off the needle. Well separated colonies are usually obtained in this way. It has been found advantageous to have plates poured on the day they are used or at most not more than 24 hours before use. Special care is taken to keep the air of the incubator moist. If the surface of the plate is dry when inoculated, or if the air of the incubator is not sufficiently moist, growth of the Pfeiffer bacillus may be unfavorably affected. The plates have been examined after 24 and again after 48 hours, always using a hand lens. In the present series particular attention has been paid to the occurrence of the Pfeiffer bacillus and of the green-producing streptococcus isolated by Mathers.[1] Other bacteria when present in noteworthy numbers have also been isolated and examined. As a rule several colonies from each plate have been circled and fished with a platinum loop to a fresh plate; if this second plate is a pure culture, appropriate diagnostic tests are made. Heated blood-agar plates on which the Pfeiffer bacillus grows with special luxuriance have been used in many cases together with the standard blood-agar plates, but their use has not been essential for the isolation of the Pfeiffer bacillus which has grown freely on the plain blood-agar medium we have used. The well known favoring influence of hemolytic colonies on the growth of the Pfeiffer bacillus has been often observed; it has also been noted that the colonies of certain nonhemolytic bacteria have a similar stimulating effect which is quite as marked. In cases in which the growth of the Pfeiffer bacillus on the first plates was scanty after 24 hours we have sometimes obtained much larger colonies by streaking the plates with one of the growth-favoring organisms and incubating for 24 hours longer. Occasionally the original plate has been too crowded for proper examination and if so, a second plate has been made from it before fishing. Two points have seemed of special importance in the successful isolation of the Pfeiffer bacillus, particularly when this organism is present in small numbers: (1) the incubation of the plates for two or three days, and (2) the use of the hand lens.

Sputum has been examined when it was possible to obtain it. Direct stains have been made and also smears on blood-agar plates in the usual way. When, as is often the case in uncomplicated influenza, sputum in washable quantity is unobtainable, we have had the patient cough directly on a blood-agar plate. Little flakes of mucus are sometimes discharged and after 18-24 hours the growth surrounding these mucous droplets is transferred with a loop to a fresh plate. In this way the Pfeiffer bacillus has been isolated several times when the other sources of examination failed to yield it.

Blood Cultures.—Blood drawn from the elbow vein (about 10 c c in each case) was added to warm broth (200-500 c c) and incubated for about a week. The observations comprised eleven of uncomplicated influenza and eight of influenza pneumonia. In the plain influenza cases blood was drawn on the 1st day in two, on the 2nd day in five, and in one each on the 3rd, 4th, 5th and 7th days; in the pneumonia following influenza the blood cultures were made, respectively, on the 4th (2), 5th (1), 6th (1), 7th (2), 8th (1) and 12th (1) days. All were sterile.

The Pfeiffer Bacillus.—The small translucent colonies on blood agar can often be identified with a high degree of certainty with the hand lens, particularly if they are numerous, in which case the heaping-up around large colonies of staphylococci and other bacteria is highly characteristic. For definite identification it has been our practice to transfer single isolated colonies to a fresh plate; if typical growth was obtained, failure to grow on plain agar together with characteristic morphology and gram-stain reaction were regarded as sufficient identification marks. A luxuriant slimy growth can be obtained on agar prepared with heated blood, but except for obtaining large quantities of material for inoculation experiments this medium presents no noteworthy advantages over the ordinary clear medium. It is not particularly well adapted for isolation.

It is of interest that a medium made without meat infusion, meat extract or peptone—simple agar dissolved in physiologic salt solution—to which 5% of blood is added in the usual way, yields a scanty but undoubted growth of the Pfeiffer bacillus.

For preserving cultures it is safest to make daily transfers, though if the air of the incubator is kept moist, the Pfeiffer bacillus can retain its vitality for a considerable period in the incubator. We have frequently obtaned growth from tube cultures that had been in the incubator for from two to three weeks. The heated blood medium is better than the ordinary blood medium for maintaining vitality.

The Mathers Coccus.—This organism was isolated by the late Captain Mathers during the influenza epidemic in September, 1918, at Camp Meade. A culture kindly furnished me by Dr. Tunnicliff possessed the characters described in her paper.[3] It resembles the ordinary mouth streptococcus in some of its characters, but the colonies on blood agar are much like those of the pneumococcus, although as a rule larger, moister and more confluent. It is gram-positive, usually with pointed ends and in pairs. It is not soluble in bile, and most strains ferment neither inulin nor mannite. Morphologically and in colony growth it is closer to the pneumococcus than to the streptococcus, but the fermentation characters are those of the ordinary mouth streptococci.

A coccus with these characters was found in a large percentage of the cases examined, not infrequently in practically pure culture especially in cultures from nasopharyngeal swabs. One hundred and eight strains obtained at different times from 44 different cases were subjected to careful examination. All were gram-positive, had the morphology described above and gave a heavy, moist, green, confluent growth on blood agar. Table 1 shows their close relationship to strains of Streptococcus buccalis (Blake's classification) isolated in this same series of cases, altho in morphology and in appearance of the growth on blood agar the difference is sharp.

[3] Jour. Amer. Med. Assn., 1918, 71, p. 1733.

TABLE 1

SHOWING THE CLOSE RELATIONSHIP BETWEEN THE DIFFERENT STRAINS

No. of Strains	Bile Solubility		Fermentation						
			Lactose		Inulin		Mannit		
	+	−	+	−	+	−	+	−	
108	0	108	105	3	4	104	5	103	Mathers' coccus
85	0	64	84	1	2	62	1	63	Streptococcus buccalis
27	27	0	27	0	22	5	22	5	Pneumococcus IV

TABLE 2

METHODS OF OBSERVATION EMPLOYED

Day of Disease	Temp.	Leukocyte Count	Pfeiffer Bacillus	Mathers' Coccus	Other Bacteria	Remarks
Case 21: (Influenza-Pneumonia)						
2	103.0	7,300	−	+	Pneumococcus IV M. catarrhalis	M. catarrhalis more abundant than any other on these dates
3	102.0	5,500	−	+	M. catarrhalis	
4	103.0	5,000	−	−	M. catarrhalis	
5	103.0	3,700	−	−	Many diphtheroids	
7	104.2	4,600	−	−	Many staphylococci	
8	104.0	4,200	−	−	Many diphtheroids and streptococci	
9	103.2	6,200	−	−	Many staphylococci	Almost pure culture of staphylococci in nasopharynx. This is rather unusual
15	100.4	9,900	+	−	Streptococcus buccalis, Pfeiffer bacillus very abundant	
18	99.4	14,400	+	+	Some diphtheroids	
21	98.4	14,200	+	+	Strep. buccalis	
26	98.0	12,900 (24th)	+	−	Strep. buccalis	
37	98.0	7,800	−	−	Strep. buccalis M. catarrhalis	
Case 24: (Uncomplicated Influenza)						
1	102.0	−	−	−	Staphylococci	
2	102.0	6,000	−	−	Diphtheroids	
3	100.6	5,400	−	−	Diphtheroids, M. catarrhalis	
5	99.0	−	+	−	M. catarrhalis	
6	99.0	8,000	+	−	B. mucosus capsulatus	
8	Discharged					
Case 66: (Uncomplicated Influenza)						
1	103.0	4,900	−	+	A few Strep. buccalis	Almost pure culture Mathers' coccus
2	100.0	−	−	+	A few Strep. buccalis	
3	100.0	−	−	+	Staphylococci	Mathers' coccus not nearly so abundant as on preceding days
5	97.6	−	+	+	Many staphylococci	
7	97.0	7,460	+	+	Few if any bacteria besides Pfeiffer bacillus and Mathers' coccus	
9	97.0	7,300	+	+	Mathers' coccus abundant	
14	Discharged					

Attempts to differentiate the Mathers coccus and S. buccalis by testing their fermentation powers on a large number of carbohydrates have given negative results. A comparison of ten strains of each gave results as follows: Positive: lactose, saccharose, maltose (1 strain of S. buccalis, negative), galactose (1 buccalis, negative; same strain negative in maltose); mannose (2 S. buccalis, negative). Negative: inulin, mannite, arabinose, raffinose, sorbite and dulcite. Type pneumococci (I, II and II) gave similar results with these carbohydrates except that inulin and mannite were fermented by all.

The methods of observation of the cases studied is illustrated in table 2.

In all, 47 cases of influenza were studied in this way. The distribution of the Pfeiffer bacillus and the Mathers coccus was as follows:

TABLE 3

DISTRIBUTION OF THE BACTERIA IN SAME EPIDEMIC

		No. Cases Examined	No. in Which Pfeiffer Bacillus Was Found	No. in Which Mathers' Coccus Was Found
*Group A-1	Uncomplicated influenza (October)	11	8	4
Group A-2	Influenza-Pneumonia (October)	8	3	8
Group B	Hospital influenza and influenza-pneumonia	17	11	8
Group C	Uncomplicated influenza (December-February)	11	8	11
		47	30 = 64%	31 = 66%

* These groups are described in the first paragraph in this paper.

Arrangement in such a numerical table has its limitations and does not give a complete picture of the findings since most of the cases in Groups A-1, A-2, and C were examined many times while nearly all of those in Group B were examined only once. Groups A-1 and A-2 are fairly comparable with one another in respect to the number of observations in each case, and so far as the examination of this limited number of cases from one localized epidemic is concerned, there is no doubt that Pfeiffer bacilli occurred more frequently and more abundantly in the uncomplicated influenza cases than in those in which pneumonia developed.

Comparison of the tabular record for Groups A-1 and C, on the other hand, might be misleading since the Pfeiffer bacillus was present in much larger numbers in the December-February cases (Group C) than in the October cases (Group A-1). Whereas, in the earlier cases Pfeiffer bacillus colonies were relatively infrequent compared with the numbers of other bacteria, in the later cases there were many plates in which the Pfeiffer bacillus and the Mathers coccus were practically the only organisms present. Considering the number of colonies

of Pfeiffer bacillus on each plate and the proportion of daily observations that were positive, the actual abundance of this organism in the upper respiratory tract was far greater in the later cases than in the cases observed during the height of the epidemic in Chicago. The contrast between the cases in Group B and Groups A-1 and A-2 (Table 3) was even more pronounced. Altho the total of examinations in the hospital cases was much smaller, the Pfeiffer bacillus was present in a relatively high proportion of the plates examined and usually in great numbers. The significance of relative abundance as disclosed by plate culture is somewhat problematical and the bearing of such facts on the rôle of the Pfeiffer bacillus must be regarded for the present as quite uncertain.

In 18 cases in which the Pfeiffer bacillus was found, examinations made on the 1st to 3rd day of the disease showed this organism present in 8, the Mathers coccus in 11 cases; in seven cases neither of these organisms were found on the first three days of the disease. In six of the ten cases in which the Pfeiffer bacillus was not found on the first three days, it was found later (See, for example, Table 2, Cases 24 and 66). The Mathers coccus, when present at all, was always found on the early days of the attack.

Other Bacteria.—The usual bacteria of the upper respiratory tract were found in most of the cases altho in greatly varying numbers, both in different individuals and in the same individuals on different days. The men in the Section B epidemic group (Table 1, Groups A-1 and A-2) harbored a far greater variety of bacteria than patients from other sources. The close contact of these men with one another during their preliminary illness and in the emergency hospital evidently favored a generous transfer of bacteria from throat to throat. The result was that at the time of our examination the variety of bacteria that had found a congenial soil was very large. Diphtheroids were especially numerous and were found richly in all the earlier cases. M. catarrhalis was also very commonly present, often in great numbers (See Table 2, Case 21). Streptococcus buccalis occurred in varying numbers, but was rarely very abundant. In many of the Section B cases in October a large gram-negative diplococcus was present which formed very delicate translucent colonies and died out readily even when transferred frequently on blood agar. It grew in the first generation very scantily on plain agar and since it did not ferment dextrose is perhaps to be regarded as belonging to the

M. catarrhalis group, altho under the conditions of our work it proved a much less vigorous organism than M. catarrhalis or than the Pfeiffer bacillus.

Pneumococci were found in ten cases, but several strains were irregular in respect to inulin and mannite fermentations. All but one (IIa) fell in Group IV on application of the agglutination test.

The Friedländer bacillus was found in four cases, in one of these in large numbers.

Hemolytic streptococci were found in seven cases, all but one of these (5) in the later stages of the epidemic; in five cases they were numerous. In one patient no hemolytic streptococci were observed during the primary attack (3 sets of examinations). Twelve days after recovery and discharge, the patient was readmitted with a temperature of 104 F. and subjective symptoms described as being very similar to those of the original attack; hemolytic streptococci were present in practically pure culture in throat and nasopharynx. This second attack or relapse was of short duration and the patient was discharged 5 days later. Bacteriologically it appeared like a new infection with an organism not originally present. The leukocyte count on the 4th day of the second attack was 14,500. A second case of the same nature was observed later.

Bacteriology of Colds, etc., During the Epidemic.—Twenty-eight cases of tonsillitis, sore throat and common cold among University students were examined, about half of these (13) while the influenza epidemic was at its height in October-November, 1918, the others in January-March, 1919, after the influenza cases had practically disappeared from the neighborhood. The organisms most commonly found on blood-agar plates were as follows:

Total cases examined	28
Mathers' coccus	15
Hemolytic streptococci	12
Pneumococcus IV	7
Pfeiffer bacillus	4

The Friedländer bacillus was found once in abundance and M. catarrhalis was found several times, though not in large numbers.

Observations on these cases were made in precisely the same manner as on the influenza cases. Typical records in Table 4 may be compared with the influenza records in Table 2.

TABLE 4

A SERIES OF TYPICAL RECORDS

Day of Disease	Temp.	Leukocyte Count	Pfeiffer Bacillus	Mathers' Coccus	Other Bacteria
Case 100 (Common Cold)					
2	104	8,900	—	—	M. catarrhalis
6	101.6	8,100	—	—	Hemolytic streptococcus
7	102	7,800	+	—	Streptococcus buccalis
Case 101 (Severe Cold, Sore Throat)					
3	102	10,500	—	—	M. catarrhalis
5	100	8,100	+	+	
6	99	7,900	+	+	Pneumococcus IV
7	...	6,000	—	+	
8	...	5,400	—	+	Streptococcus buccalis
13	100	10,200	—	—	Streptococcus buccalis
14	99	7,600	—	+	Streptococcus buccalis
Case 103					
1	98	12,900	—	+	Pneumococcus IV. S. buccalis
2	98	.....	—	+	Pneum. IV, S. buccalis, M. cat.
4	98	10,300	—	+	Pneum. IV, S. buccalis, M. cat.
5	98	7,800	—	+	S. buccalis, M. catarrhalis
7	98	6,600	—	+	Pneum. IV, S. buccalis, M. cat.
Case 111* (Tonsillitis)					
2	102	9,700	—	+	Hemolytic streptococci (almost pure culture, very few other colonies)
4	...	13,400	—	+	S. buccalis

* Case 111 was one of four cases of tonsillitis developing at the same time, and all with a history of exposure at a party 18-24 hours before attack. All four cases showed leukocytosis during the first 4 days of the attack, the maximum ranging from 13,400 in one case to 20,800 in another. Three of the cases showed a relative increase of the neutrophils (82 to 86%). The bacteriologic picture as shown in throat and nasopharynx swabs was remarkably similar. Hemolytic streptococci were very abundant on all plates and were by far the predominating organisms. In one case pneumococcus (Type IV) was present in small numbers in one examination, but in the other only a few colonies of the Mathers streptococcus and of S. buccalis were found in addition to the hemolytic streptococci. Between the first and second examinations two throat treatments with methylene blue were given, but the character of the flora of nasopharynx and throat was not appreciably altered by this procedure.

The clinical picture of these common endemic respiratory tract infections was distinctly different from that of epidemic influenza altho many of the symptoms were similar and the attack at first was frequently regarded by the patient and others as influenza. Headache, dizziness and pain in the joints and limbs were common accompaniments of these cases and in some instances the onset was sudden. On the other hand, sore throat was much more frequent and pronounced than in the cases of epidemic influenza and patches were often observed on the mucous membrane. In the majority of cases the temperature did not run above 102 F. and the fever was not prolonged beyond 24-48 hours. The leukocyte count was high in nearly all these cases. Thirty-nine leukocyte counts made in 20 cases, usually on the

2 days of the height of the attack gave an average of 11,500. The range was as follows:

Above 10,000	22
7,000-10,000	11
Below 7,000	6

In only one case was the leukocyte count similar to that observed in the majority of cases of true influenza. In this case (107) the leukocytes were: 3rd day, 6,700; 4th, 4,500; 5th, 5,200; 7th, 5,100. The symptoms were not unambiguous: headache, pain in back, no sore throat, no cough, no nosebleed. The temperature was not high (2nd day, 101.8) and quickly subsided (3rd day, 98.6) and none of the symptoms were severe. The predominating organism on the 3rd, 4th and 5th day was the Mathers coccus altho some Pneumococci (Type IV) were found on the 4th and 5th days. On the 7th day many Friedländer bacilli were found. This case may have been one of light influenza, but there were no other evident cases in the neighborhood or among the associates of (107) at this time (Feb. 2-8, 1919).

In comparing the October-November cases of cold with those in January-March the most conspicious difference was the much larger number of cases with hemolytic streptococci and with pneumococci (Type IV) in the latter group.

TABLE 5

COMPARISONS MADE AT DIFFERENT PERIODS DURING THE EPIDEMIC

	Height of Influenza Epidemic Oct.-Nov., 1918	After Subsidence of Influenza Epidemic Jan.-March, 1919
No. cases	13	15
Hemolytic streptococci	2	10
Pneumococcus IV	1	6
Mathers coccus	4	11
Pfeiffer bacillus	1	3

The richer flora in the second group of cases may be connected with the greater severity of these cases and this in turn, dependent on the season of year. At all events the relatively mild cases of common respiratory tract infection that were examined during the height of the influenza epidemic did not harbor the Pfeiffer bacillus in nearly as high proportion as did the influenza cases examined at the same time; neither did they, except in a few cases, harbor in abundance hemolytic streptococci or the Mathers' coccus. In several cases the only organ-

ism growing in any considerable numbers on blood-agar plates was the common mouth streptococcus (S. buccalis).

The association of large numbers of hemolytic streptococci (var. beta) with cases of tonsillitis and generally with cases of severe throat inflammation was markedly evident in this series. This corresponds with the relative scarcity of this organism in the cases of true influenza in which, as a rule, sore throat was not observed.

Leukocyte Counts.—In the course of these observations leukocyte counts were made as a rule on the same days when throat swabs were taken. The average counts in simple influenza showed a leukopenia as recorded in Table 6. The term simple influenza is here used to designate those cases not showing signs of clinical pneumonia. The patients usually regained a normal condition by the end of the first week, although with the ordinary aftermath of weakness.

TABLE 6

LEUKOCYTE COUNTS: SIMPLE INFLUENZA

Days	Cases	Total Leukocytes	Polymorphonuclears, per Cent.	Lymphocytes, per Cent.	Large Mononuclears and Transitionals, per Cent.
2 and 3	13	6,100	63	28	9
4 and 5	9	4,900	62	33	5
6 and 7	6	6,100	59	32	9
8–12	10	7,500	66	25	9
12–30 (after full recovery and discharge)	8	9,100	60	34	6

Eosinophils were generally absent during the attack.

Individual cases sometimes showed considerable deviation from the averages. In one case the lowest count was not reached until the 8th day of the disease (4,300). Several cases without any apparent complication gave leukocyte counts of over 10,000 for a week or more after seemingly complete recovery. In general, the counts ran rather high for some time after the patient was able to return to his ordinary avocation.

Similar observations on the influenza cases in which clinical pneumonia developed showed a drop in the number of leukocytes similar to that recorded above, but after a longer or shorter interval this was followed in each of the cases here observed by a moderate leukocytosis (14,000-15,000) which, however, in one case (Cf. 21, Table 2) did not appear until about the 15th day. The differential ratio was not appreci-

ably altered in these cases. Quite different is the normal leukocyte count in ordinary colds, tonsillar inflammation, etc.

TABLE 7
LEUKOCYTE COUNTS: COLDS, ETC.

Days	Cases	Total Leukocytes	Polymorphonuclears, per Cent.	Lymphocytes, per Cent.	Large Mononuclears and Transitionals, per Cent.
1, 2 and 3	22	12,600	73	22	5
4 and 5	8	11,600	74	20	6
6 and 7	7	8,800	69	25	6
8-14	3	7,200	68	25	7

Comparison with Table 6 brings out very plainly the average differences in total leukocyte counts. Whether the slight divergences in the differential count that appear in these figures have significance will need a larger number of observations to determine. As already stated several cases have been observed in the course of this study which clinically seemed more like colds than like true influenza, but which had a low leukocyte count throughout. In the absence of any definite diagnostic criterion the relationship of these infections to true influenza must remain uncertain.

SUMMARY

The bacteriologic picture in influenza is not a uniform one so far as nose and throat flora is concerned. The ordinary methods of cultivation with blood-agar plates show marked differences in individual cases. Groups of individuals who have been in more or less intimate contact with one another may harbor very similar assemblages of micro-organisms, but differ from other groups examined at the same time. The variations in respiratory tract flora reported by many observers during the progress of an influenza epidemic are doubtless, in part, group differences.

Daily examination of a number of selected typical cases, mild and severe, have shown no one organism constantly demonstrable in large numbers by the methods employed. The two organisms most commonly and abundantly present in this series were the Pfeiffer bacillus and the diplococcus or streptococcus found by Mathers at Camp Meade.*

* This is apparently very similar to, if not identical with, the organism described by Zingher (Jour. Amer. Med. Assn., 1919, 72, p. 1020).

The Pfeiffer bacillus was found in 64% of the influenza cases examined between October, 1918, and February, 1919. It was present in much larger numbers in the throats of the patients examined toward the end of the outbreak. Several cases did not come under observation until clinical pneumonia had developed, and in these cases the Pfeiffer bacillus was not found as frequently as in the "uncomplicated" cases. Other cases for various reasons could be examined only once or twice, and the findings are not strictly comparable with those in which daily examinations were carried out. The percentage of positive findings would probably have increased if all patients had been subjected to examination throughout the course of the attack. The relative abundance of the Pfeiffer bacillus varied greatly. In some cases it was the predominant organism, in others only a few colonies could be found, no more than in normal throats. It did not often happen in this series that hemolytic streptococci (var. beta) and the Pfeiffer bacillus were both present in large numbers. On the other hand, the Pfeiffer bacillus and the Mathers coccus often had the field almost to themselves. In a few cases the Pfeiffer bacillus was present in such overwhelming numbers in cultures from nose, nasopharynx and throat that participation in a pathologic process was strongly suggested. These cases, however, did not differ clinically in any appreciable way from other cases in which the Pfeiffer bacillus was found scantily.

The Mathers coccus was found about as frequently and abundantly as the Pfeiffer bacillus, altho its occurrence was quite independent of that of the latter. Its association with the pneumonia cases seemed to be closer than that of the Pfeiffer bacillus, but it was also found in all the later cases of simple influenza. Variations in the abundance of this organism were quite as marked as were those of the Pfeiffer bacillus, and no relation could be demonstrated between these findings and the characters of the cases. Practically pure cultures of the Mathers coccus were obtained from the nasopharynx of some patients.

Comparison of the true influenza cases with colds and tonsillar infections showed that the general leukopenia of the influenza cases could be contrasted with the general leukocytosis of the others. Blood counts made during the first 4 or 5 days of the attack practically invariably showed at some time a leukopenia in the influenza cases and a leukocytosis in the others. The chief differences in the bacterial findings were the relative infrequency of the Pfeiffer bacillus in the colds, etc. (14%), and the relatively high proportion of hemolytic

streptococci (var. beta). The Mathers coccus was present in about the same proportion of cases as in influenza.

The pneumococcus was found in about 20% of the influenza cases (10:47), and in a slightly larger proportion in the cases of rhinitis and tonsillitis (7:28). No special search was made for these organisms, and it is probable that positive findings would have been increased somewhat if mice inoculation could have been made whenever throat swabs were taken. Washed sputum, when procurable, was inoculated into mice, but the pneumococcus was not invariably isolated. In two cases as much as 2 c c of washed sputum from influenza patients injected intraperitoneally did not lead to the death of the mouse. The pneumococci isolated from both influenza and rhinitis cases were all Type IV except one strain (IIa).

M. catarrhalis, the Friedländer bacillus and an unidentified gram-negative diplococcus were found, at times, in large numbers in the throat, nose and nasopharynx of influenza cases. Staphylococci were also sometimes present in great abundance.

Two observations on suspected cases of "recurrence" or "second attack" have shown the presence in the throat of organisms (hemolytic streptococci) not found during the original attack. Both recurrences presented some of the clinical symptoms of influenza, but had a moderate leukocytosis. A third case of suspected "recurrence" likewise showed hemolytic streptococci in the throat and slight leukocytosis, but this patient had not been under our observation during the primary attack. It seems probable that an alleged "second attack" of influenza occurring within a few weeks of the original attack is at least in some cases a new infection with another organism.

The observations carried out by the aerobic blood-agar plate method and recorded in this paper have not shown the predominance or constant presence of any one organism in the upper respiratory tract of influenza patients. The Pfeiffer bacillus, however, has been more conspicuous than any other organism, particularly in comparison with its relative infrequency in cases of rhinitis and tonsillitis examined during the same epidemic period.